REMARKS

Applicant has added claims 132-136 directed to preferred embodiments of the invention. These claims are supported at pages 29-30 and Example 14 at pages 49-51. As such, these amendments do not constitute new matter, and their entry is respectfully requested.

Applicant appreciates the Examiner's indication that claim 117 would be allowable if rewritten in independent form.

The Examiner rejected claims 111-112, 114-116, 118, 120-121 and 126-131 pursuant to 35 U.S.C. § 102(b), over *Gram et al.*

Applicant respectfully submits that this rejection should be withdrawn for the following reasons.

Gram et al. does not disclose in-mass transfer as described in the present invention. The Examiner points to the methods section on page 3577 of *Gram et al.* This section discusses the generation of a first library of naïve immunoglobulin μ/κ library in pComb8 phage vector. In the next paragraph, "**Panning**", this first library is subjected to selection (panning) and isolation of clones that bind specifically to progesterone. It is only these progesterone-binding clones (page 3577, second column, line 6 to 7 "progesterone binders") that are subjected to mutagenesis in order to obtain *in vitro* affinity matured antibodies. Specifically **three** clones (PgA11, PgB6, and PgF1) form the basis of the mutagenesis experiments: From the results section (page 3578, first column, last paragraph) it is clear that the three clones, PgA11, PgB6 and PgF1 were isolated from the panning rounds for further characterization, such as cross-reactivity and apparent binding constants, and from page 3579 line 12 it is clear that only these three clones were used for mutagenesis.

The Methods section on page 3577 under "**ScFv-gIII Fusion**" describes the transfer between vectors of the sequences encoding the three characterized clones mentioned above stating:

> "The V_H and V_L segments of the progesterone binders were amplified by PCR using the ... [*two primer pairs*]..., respectively". [(Emphasis added) (lines 6-11, second column)]

Thus, the V_H and V_L sequences are amplified **separately** in separate reactions yielding two separate V_H and V_L segments for each of the three antibody clones. The section goes on to teach that:

> "The V_H and V_L Fv PCR fragments were digested with XhoI/XbaI and SacI/SpeI, respectively, and subsequently inserted into ScpComb3". [p. 3577, lines 13-15, second column (emphasis added)]

Thus, the V_H and V_L segments are digested separately before they are ligated into ScpComb3 in separate reactions, one segment after the other.

The fact that the V_H and V_L segments are isolated from the pComb8 vector by separate PCR reactions, digested separately and ligated separately, as described therein above, clearly teach that the V_H and V_L sequences of each clone are transferred separately, and **not as pairs contained in single segments**, as required by claim 111. This is not an "in-mass" transfer. Rather it is a specific set of transfers.

The fact that the V_H and V_L sequences are transferred independently from each other and not as a pair, teaches that they are transferred from single clones and not from a mixture of the three clones (a "library"). Using transfer in-mass from a library would result in nine combinations of V_H and V_L sequences, out of which six would be new, unscreened combinations. The statement "For mutagenesis, a mixture of the three ScFv templates was subjected to error-prone PCR amplification" (page 3579 line 12), further confirms that only three clones were generated by the transfer from pComb8 to ScpComb3 and not nine, in agreement with the fact that V_H and V_L sequences were transferred from single clones.

Indeed, if in-mass transfer from a library was used rather than transfer from individual clones, the six new combinations of V_H and V_L sequences would likely be non-functional and irrelevant. This problem elegantly exemplifies how the present invention solves a fundamental problem of random scrambling between heavy chains and light chains of different antibodies, which would occur during conventional transfer as disclosed by *Gram et al.* if the transfer was performed from a polyclonal library and not from single clones. The present invention teaches that during the mass transfer the V_H and V_L sequences are kept together in a single segment, thereby permitting V_H and V_L combinations selected *in vitro* for their binding properties or natural *in vivo* occurring pairs of V_H and V_L, to be transferred in-mass from one vector to another without creating numerous V_H and V_L permutations (see page 8 of the specification, last

paragraph which discusses the importance of keeping the proper V_H or V_L sequences together with respect to natural *in vivo* occurring V_H and V_L combinations).

Gram et al. does not disclose the transfer of pairs of variable regions contained in a single segment from a first library to a second vector, since the transfer is not performed from a library, and importantly neither as pairs of variable regions. The purpose of *Gram et al.* is **not to generate a diverse library**, but to generate single clones encoding *in vitro* affinity matured mouse monoclonal antibodies from a naïve library. Thus, it does not teach the diverse composition required by the claims.

In particular, claim 111 discloses mass transfer of joined V_H and V_L segments, and claim 115 specify that the individual library members are not characterized prior to transfer in contrast to *Gram et al.* Claim 121 specifies that the library encode full-length receptor proteins, which is not taught by *Gram et al.* as acknowledged by the Examiner in point 7 of the Office Action. Claims 126 to 133, and 135-136 specify the transfer of a mixture of at least ten different variable region sequences, compared to the three clones transferred individually in *Gram et al.* Claim 134 specifies that diversity is reduced by less than 10% in contrast to *Gram et al.* which reduces the naïve library to only three clones. Accordingly, the rejection should be withdrawn.

Claims 111-112, 114-116, 118-121 and 123-131 were rejected pursuant to 35 USC § 103(a) over *Gram et al.* in view of *Bender et al.*

Applicants respectfully submit that this rejection should be withdrawn for the following reasons.

As explained above, *Gram et al.* does not describe the generation of a diverse library obtained by transfer in-mass. The addition of *Bender et al.* does not overcome this deficiency. Further, it is not logical to combine *Bender et al.* with *Gram et al.*. *Gram et al.* specifically teaches the generation of single chain constructs. On page 3578 bridging page 3579 it reads "To target the mutations by error-prone PCR specifically and only to the V regions, we created a ScFv-gIII fusion plasmid [ScpComb3]". This is essential to what *Gram et al.* was doing because the use of Fab or full-length constructs would result in mutations in regions not subjected to somatic mutations in vivo. Thus, the generation of full-length antibodies as taught in *Bender et al.* would be contradictory to the purpose of the transfer presented in *Gram et al.*

In contrast, *Bender et al.* discusses full length, fully human antibodies as preferable for therapy. Thus, the technique described, aims to substitute previous antibody technologies based

on sequences derived from mice, such as hybridomas and humanized antibodies (page 74 second column line 4 and line 15), with fully human full-length antibodies. In contrast, *Gram et al.* is concerned with the very generation of *in vitro* mouse antibodies that *Bender et al.* teaches away from. Accordingly, one of ordinary skill in the art would not combine *Gram et al.* with the technique presented by *Bender et al.*

Even assuming arguendo that one would combine the two teachings, they would not generate a diverse library as claimed in claim 111 suitable for expression of polyclonal antibodies.

The vector system described by *Bender et al.* is only useful for expression of monoclonal antibodies, because the V_H and V_L chains are located in two separate vectors (page 75 second column first line in the results section and figure 1). If one were to transfer a diverse library of pairs of variable regions from a first vector into the vector system described by *Bender et al.* it would require that all the V_H sequences were isolated from the library and inserted into the lower vector in figure 1, and in a separate step all the V_L sequences should be transferred to the upper vector of figure 1 (page 76 *Bender et al.*). The result would be a library, where pairs of V_H and V_L sequences, previously screened for a desired binding specificity, would now be separated, because V_H and V_L cannot be transferred **as pairs contained in a single segment**. Thus, one would lose the desired V_H and V_L combinations and the resultant antibodies would most likely not have the desired binding specificity. This as explained above, is because the combination of *Bender et al.* with *Gram et al.* does not teach or suggest the claimed in-mass transfer using a nucleic acid segment encoding an associated pair of V_H and V_L segments. Accordingly the rejection of the claims should be withdrawn.

In view of the foregoing, all claims are in condition for allowance. Early and favorable action is requested.

Respectfully submitted,

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